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Serotonin metabolism in rat mesangial cells: Involvement of a serotonin transporter and monoamine oxidase A

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Background. Serotonin is one of the factors regulating mesangial cell proliferation, and convergent evidence supports its involvement in the development of glomerulonephritis. In this study, we identified a serotonin transporter and the amine-degrading enzyme monoamine oxidases (MAOs) in mesangial cells, and we studied their involvement in serotonin degradation.

Methods. MAOs were characterized in membrane preparations and intact mesangial cells by enzyme assay using [^{14}C]5-hydroxytryptamine and [^{14}C]β-phenylethylamine as specific substrates for MAO-A and MAO-B, respectively, and by Western blot analysis. The expression of a serotonin transporter was determined by [^{14}C]5-hydroxytryptamine uptake experiments and Western blot. Mesangial cell proliferation was measured by BrdU incorporation.

Results. Quantitation of the MAO isoforms by enzyme assay and Western blot analysis showed that MAO-A was largely predominant in mesangial cells, accounting for approximately 90% of the total enzyme population. The MAO substrate [^{14}C]serotonin was transported into mesangial cells by a saturable uptake system (V_{max} 310 ± 36 pmol/30 min/mg protein; K_m 5.9 ± 1.4 μM) displaying the pharmacological properties of a serotonin transporter. The expression of a serotonin transporter was confirmed by Western blot analysis. MAO activity measured in intact cells showed that after accumulation into mesangial cells, [^{14}C]serotonin was metabolized by MAO-A. Finally, serotonin-mediated mesangial cell proliferation was significantly increased after irreversible MAO inhibition.

Conclusions. Our results suggest that serotonin concentration and function in glomeruli may be regulated in part by its transport into mesangial cells and degradation by MAO-A.

Mesangial cells (MCs) are a major constituent of renal glomerulus. These smooth muscle-like cells play a critical role in the regulation of glomerular filtration rate [1] and participate in the development of functional and morphological glomerular abnormalities in inflammatory processes [2]. The function of MCs is regulated by a variety of mediators, including biogenic amines, angiotensin II, bradykinin, growth factors, and cytokines [3]. Some of these factors, including dopamine [4], angiotensin II [5], bradykinin [6], and serotonin [7], are mostly involved in MC contraction. Moreover, serotonin [8], cytokines, and growth factors [9] play a particularly important role in the modification of MC function occurring in glomerulonephritis.

Several studies showed that serotonin is a potent mitogen factor of MCs [7, 10, 11], and this mitogen activity may participate in the development of glomerulonephritis. Part of the serotonin mitogen activity is related to a receptor-mediated second-messenger generation [12]. In addition, it has been recently suggested that part of the effect on cell proliferation requires serotonin uptake into the cells and, therefore, could occur independently of membrane receptor stimulation [13, 14]. Most of the serotonin acting on MCs is released by platelets infiltrating the site of glomerular injury. Recent studies showed that serotonin can be produced by the renal proximal tubule and secreted at the basolateral side, where it acts as an autocrine factor [15]. It is conceivable that part of the serotonin released from the proximal tubule may reach the glomerulus to regulate MC activities under physiological conditions.

Extracellular availability of biogenic amines (that is, serotonin, norepinephrine, epinephrine, and dopamine) depends in part on their degradation rate by the mitochondrial enzyme monoamine oxidases (MAOs) [16, 17]. Currently, two MAO isoforms, encoded by separate genes sharing a common intron/exon organization [18], have been identified based on substrate specificity: MAO-A preferentially metabolizes serotonin and kynuramine, whereas MAO-B has a greater affinity for phenylethylamine and benzylamine [19]. The two MAO

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isoforms can be also differentiated according to their inhibition by synthetic compounds: clorgyline and moclobemide for MAO-A and selegiline and lazabemide for MAO-B [20]. Several studies showed that the kidney contains one of the highest MAO activities of the body [21, 22]. Although both MAO-A and MAO-B have been identified in cortex and medulla, their precise localization in the different portions of the nephron has not been investigated with the exception to the proximal tubule [23, 24].

As MAO are mitochondrial enzymes, serotonin needs to be transported into the cells to be metabolized. The mechanism of intracellular serotonin transport has been extensively investigated in the central nervous system. In presynaptic terminals and glial cells [25], serotonin uptake is mediated by a specific serotonin transporter (SERT). This transporter, which in human and rat contains 630 amino acids, displays a very low affinity for other biogenic amines (for example, norepinephrine and dopamine) and is selectively inhibited by the antidepressive drugs clomipramine and fluoxetine [26–28]. Several studies have shown that the SERT is also expressed in peripheral organs such as lung, placenta [29], and adrenal medulla [25, 30], where it seems to regulate serotonin clearance. Currently, the expression of a SERT in the kidney has not been reported.

In this study, using different approaches, we show, for the first time, that MCs contain under normal culture conditions a functional SERT and the serotonin-metabolizing enzyme MAO-A. In addition, we demonstrate that serotonin-mediated MC proliferation was significantly increased after irreversible MAO inhibition. These data suggest that SERT and MAO-A may be one of the factors regulating the serotonin level and its functional activity in mesangium.

METHODS

Cell culture

Rat MCs were isolated and cultured as described previously by Edmond et al [31]. Briefly, primary MCs were obtained as outgrowths of collagenase-digested glomeruli. Glomerular explants were allowed to grow to confluence in RPMI supplemented with 1 mM sodium pyruvate, 100 units of penicillin, 100 μg/ml streptomycin, and 15% fetal calf serum (FCS). Cells were used three weeks after the beginning of culture or were treated with trypsin/ethylenediaminetetraacetic acid (EDTA) and transferred to new culture dishes in the same complete medium. All experiments were performed using confluent primary cells and cells from passages four through seven. Cells were identified as MCs according to the following criteria, as previously described [31]: (*a*) spindle shaped morphology, (*b*) resistance to puromycin (unlike epithelial cells), (*c*) negative staining for factor VIII (unlike endothelial cells), (*d*) the ability to grow on D-valine–containing medium (unlike fibroblasts), (*e*) positive staining with anti–Thy1-1 antibody, (*f*) the presence of actin filament revealed by NBD-phallacidin, (*g*) the presence of α smooth muscle actin (α-SMA). The expression of α-SMA received much attention. Because α-SMA is not expressed *in vivo* as well as in three-dimensional culture but is expressed in MCs under normal development and in experimental and human glomerular diseases, the expression of α-SMA is considered a marker of MC activation. Under our basal conditions, MC expressed α-SMA and serum deprivation for 48 hours, which is the maximum time tested, did not change the expression of the protein as assessed by immunofluorescence. Moreover, within the number of passages used (between passages 4 through 7) in our experiments, the doubling time of the cell cycle remained unchanged.

Amine oxidase activity

Monoamine oxidase activity was measured by the slightly modified method of Yu [32]. Cells were harvested in sodium phosphate buffer (50 μM, pH 7.4), supplemented by protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 10 μg/ml bacitracin, and 2 μg/ml soybean trypsin inhibitor). Crude extracts of rat MCs were incubated at 37°C for 20 minutes in a final volume of 250 μl of sodium phosphate buffer with a required concentration of [^{14}C]5-hydroxytryptamine (25 to 400 μM) or [^{14}C]β-phenylethylamine ([^{14}C]PEA; 0.75 to 160 μM) as specific substrate for MAO-A and MAO-B, respectively. Pargyline (10^{-5} M) was used to measure nonspecific MAO activities. The protein content varied between 100 and 200 μg/assay tube. After 20 minutes, the reactions were stopped by adding 1 ml of 2 M HCl and 2 ml of extraction solvent (toluene/ethyl acetate vol/vol). After vigorous mixing and centrifugation, 1 ml aliquot of the organic phase was counted in a liquid scintillation counter (Packard 1900 TR; Packard Instruments, Meriden, CT, USA). MAO activity was expressed as pmol of oxidized substrate during one minute of incubation per milligram of protein.

Western blot analyses

Homogenate extracts were solubilized in loading buffer [60 mM Tris-HCl, pH 6.8, containing 2% sodium dodecyl sulfate (SDS), 10% glycerol, 1% β-mercaptoethanol, and 0.05% bromophenol blue] at 100°C for five minutes and subjected to 9% SDS-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes with a semidry electroblotter (Trans-blot; Bio-Rad, Richmond, CA, USA) for one hour at 450 mA. The blots were saturated with 5% nonfat dried milk for MAO immunoblot or with 0.1% casein for SERT immunoblot in wash buffer [phosphate-buffered saline (PBS), pH 7.5 (Dulbecco, Seromed), and 0.1%

Tween 20) overnight at 4°C. Then the blots were washed twice and incubated for one hour at room temperature with a rabbit polyclonal antisera to MAO-A and MAO-B [31] or a goat polyclonal antisera directed against SERT (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The rabbit polyclonal antisera were obtained by immunization with the peptide TNGGQERKFVGGSGQ corresponding to amino acids 211 to 225 in MAO-A and 202 to 216 in MAO-B. After washing, blots were incubated with peroxidase-labeled antirabbit or antigoat for 40 minutes. Bound antibodies were detected using enhanced chemiluminescence (Amersham Life Sciences, Arlington Heights, IL, USA) and exposure to Amersham Hyperfilm™-MP film. Immunoreactive proteins were quantitated using an ImageQuant Software (Molecular Dynamics, Sunnyvale, CA, USA).

[^{14}C]serotonin uptake and metabolism in mesangial cells

Cells were preincubated at 37°C for 20 minutes with Hank's buffer containing 1 mM L-ascorbic acid and with or without 10 μM of pargyline to inhibit MAOs. Subsequently, the cells were incubated with an increasing [^{14}C]5 hydroxytryptamine ([^{14}C]5HT) concentration (100 nM to 10 μM). Specific uptake was defined as the fraction of total uptake inhibited by 10 μM of clomipramine and was expressed as pmol of [^{14}C]5HT accumulated per mg of protein during 1 or 30 minutes of incubation. Transport inhibitors were present during the preincubation and incubation period. At the end of incubation, the medium was removed to measure extracellular serotonin metabolites. Cells were rinsed four times with 4 ml ice-cold Hank's buffer and were then solubilized with 5 mM Tris-HCl, 0.1% Triton X-100, pH 7.4. Radioactivity was measured by liquid scintillation counting (Packard 1900 TR).

Serotonin metabolism in intact cells was evaluated by the concomitant measure of intracellular [^{14}C]5HT accumulation and the extracellular release of [^{14}C]5HT metabolites. Uptake experiments were performed as described earlier in this article without irreversible MAO inhibition. Extracellular [^{14}C]5HT metabolites produced by MAOs were measured by an adaptation of the original method described by Wurtman and Axelrod [33]. Briefly, metabolites were extracted from extracellular incubation medium by 2 ml of toluene/ethyl acetate (vol/vol). After vigorous mixing, a 1 ml aliquot of extraction solvent was counted in a liquid scintillation counter (Packard 1900 TR).

BrdU incorporation

Rat MCs were used between passages 5 and 7. Cells were seeded into 96-well microtiter plate in RPMI 15% FCS. After 48 hours, the growth medium was replaced by RPMI without FCS during two hours. Cells were incubated for 20 hours with indicated concentrations of 5HT with or without pargyline in RPMI 0.5% FCS. BrdU was added during four hours. Cells were harvested, and cellular proliferation was assessed by BrdU incorporation using a colorimetric immunoassay (Biotrak; Amersham).

Protein determination

Proteins were measured by BioRad assay (BioRad, Ivry/Seine, France) using γ-globulins as the standard.

Statistical analysis

Monoamine oxidase and serotonin transport steady-state kinetic parameters, IC_{50} of inhibition of enzyme activity, and IC_{50} of inhibition of serotonin transport were evaluated using nonlinear square curve-fitting procedures (Prism™; GraphPad, San Diego, CA, USA). The statistical comparison of the results was obtained by using one-way analysis of variance followed by the Newman-Keuls multiple comparison test as well as the Student's unpaired *t*-test. Results are expressed as means ± SEM.

Materials

[^{14}C]serotonin (57.8 Ci/mol), [^{14}C]β-phenyletylamine (57 Ci/mol), and polyvinylidene difluoride membranes were purchased from DuPont NEN (Le Blanc Mesnil, France). SERT antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Acrylamide, bisacrylamide, and Tween 20 were purchased from Bio-Rad. Clomipramine was provided by Research Biochemichals Int. (Natick, MA, USA). Fluoxetine was purchased from Tocris Cookson (Bristol, UK). Disprocynium 24 was a generous gift from Dr. Edgard Schomïg (Heidelberg, Germany). All remaining drugs and chemicals were purchased from Sigma (Paris, France).

RESULTS

Monoamine oxidase-A and monoamine oxidase-B activity in rat mesangial cells

Monoamine oxidases were identified and characterized by enzyme assay using [^{14}C]5HT and [^{14}C]PEA as substrates for MAO-A and MAO-B, respectively, as well as by Western blot analysis. Velocity versus substrate concentration experiments showed that MCs oxidized both [^{14}C]5HT (V_{max} 95 ± 17 pmol/min/mg protein; K_m 67 ± 7 μM) and [^{14}C]PEA (V_{max} 51 ± 3 pmol/min/mg protein; K_m 46 ± 7 μM; Fig. 1). Inhibition of enzyme activities using subtype selective MAO inhibitors showed that, as expected, [^{14}C]5HT oxidation was inhibited by the MAO-A inhibitor clorgyline (IC_{50} 0.2 ± 0.05 nM), and it was poorly affected by high concentrations of the MAO-B inhibitor selegiline (IC_{50} 2.5 ± 1 μM; Fig. 2A). Studies on the inhibition of [^{14}C]PEA oxidation supplied unexpected results. Indeed, the inhibition curve of

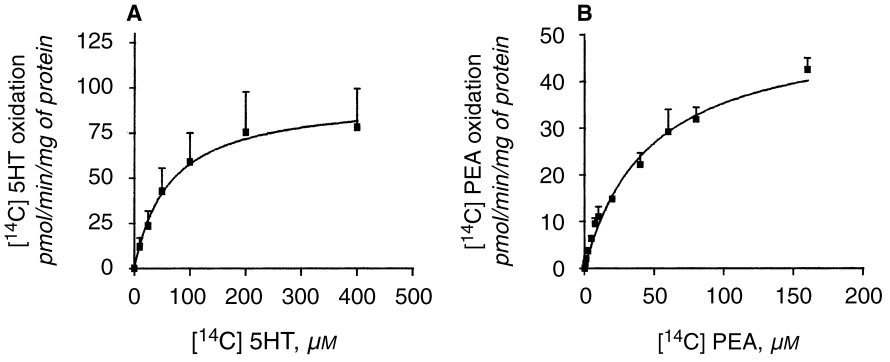

Fig. 1. Concentration-dependent oxidation of the monoamine oxidase (MAO) substrates [^{14}C]5HT (*A*) and [^{14}C]PEA (*B*) in rat mesangial cells. Cell extracts were incubated with increasing concentrations of the appropriate radioactive substrate. The curves represent the specific enzyme activity, as defined by the difference between the substrate oxidation measured in the absence or presence of the irreversible MAO inhibitor pargyline (10^{-5} M). The nonspecific activities represented 10 to 15% of the total substrate oxidation. Each point represents the mean ± SEM of four preparations.

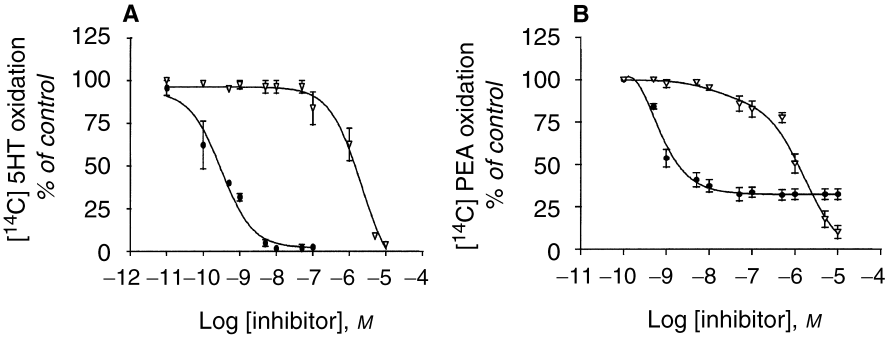

Fig. 2. Inhibition of monoamine oxidase (MAO) activity in rat mesangial cell extracts. Crude extracts of mesangial cells were incubated with [^{14}C]5HT 100 μM (*A*) or [^{14}C]PEA 20 μM (*B*) in the absence or presence of increasing concentrations of clorgyline (MAO-A inhibitor) (●) or selegiline (MAO-B inhibitor) (▽). Each point is the mean ± SEM of three to seven preparations.

[^{14}C]PEA oxidation by the MAO-B inhibitor selegiline was biphasic, that is, only 15% of the enzyme activity was inhibited by selegiline concentrations compatible with those reported for inhibition of MAO-B. In contrast, the remaining 85% of [^{14}C]PEA degradation was poorly affected by selegiline and was inhibited by the MAO-A inhibitor clorgiline with an IC_{50} close to that observed for inhibition of [^{14}C]5HT oxidation (Fig. 2B).

Western blot analysis of monoamine oxidase-A and monoamine oxidase-B expressed in rat renal cortex and mesangial cells

As enzyme assays may not provide an accurate quantitation of the MAO isoforms expressed in a given tissue [34], we determined the relative expression of MAO-A and MAO-B in MCs by Western blot analysis using an antiserum obtained from rabbit immunized with a peptide common to both MAO-A and MAO-B. The specificity of the antibody was shown by the following findings: (*a*) The antiserum revealed immunoreactive bands corresponding to MAO-A and MAO-B in mitochondria from yeast transformed with recombinant cDNA encoding for human placenta MAO-A and human liver MAO-B but not from wild-type yeasts (data not shown). (*b*) This antiserum allowed the identification and the relative quantitation of the two MAO isoforms in various human, rabbit, and rat tissues [23, 35, 36]. (*c*) We have recently

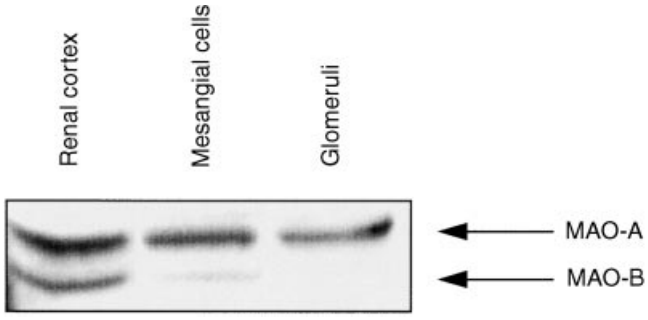

Fig. 3. Western blot of rat renal cortex, mesangial cells, and glomeruli using an anti–MAO-A/MAO-B antiserum. Crude homogenates from renal cortex (30 μg), mesangial cells (50 μg), and glomeruli (50 μg) were electrophoresed on a 9% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. The blot was incubated with a rabbit anti–MAO-A/B polyclonal antiserum. Immunoreactive proteins were identified as described in the **Methods** section. The arrows indicate proteins with molecular masses of approximately 61 and 55 kDa, corresponding to MAO-A and MAO-B, respectively. Data are representative of four experiments.

shown that, in three different cell types, the relative expression of MAO-A and MAO-B determined by Western blot corresponds to that determined by enzyme assay and measure of MAO-dependent hydrogen-peroxide generation [37]. As shown in Figure 3, Western blot analysis performed in the renal cortex revealed two pro-

teins with the apparent molecular weight expected for MAO-A and MAO-B (approximately 61 and 55 kDa, respectively). The relative abundance of MAO-A and MAO-B, estimated by densitometry, was, respectively, 60 and 40% of the total amount of the MAO immunoreactive bands. These data are in agreement with previous results obtained by enzyme assays using specific MAO-A and MAO-B substrates. The relative amount of each MAO isoform was different in glomeruli and isolated MCs as compared with renal cortex. Indeed, in both glomeruli and MCs, the approximate 61 kDa protein corresponding to MAO-A was largely predominant (Fig. 3). Quantitation of the relative amount of each enzyme isoform showed that, in MCs, MAO-A represented approximately 90% of total amount of immunoreactive proteins. Thus, both Western blot analysis and enzyme assays show that MCs contain mainly MAO-A. The results obtained in inhibition of enzyme activity indicate that in MCs, MAO-A is also able to metabolize the classic MAO-B substrate PEA.

Serotonin uptake by rat mesangial cells

Monoamine oxidase A represents one of the major metabolic pathway for serotonin degradation. As metabolism by mitochondrial MAO requires serotonin uptake into the cell, we next investigated whether a SERT was expressed in rat MCs. With this attempt, we first measured [^{14}C]5HT uptake in isolated MCs. In order to avoid the interference of serotonin degradation on uptake parameters, experiments were performed after MAO inactivation by the irreversible inhibitor pargyline. Cell incubation with 200 nM [^{14}C]5HT led to a time-dependent uptake that reached the maximum at 30 minutes and remained stable at least up to 90 minutes (Fig. 4A). The nonspecific uptake was defined in the presence of 10^{-5} M clomipramine, a potent inhibitor of SERT, and represented 20% of the total [^{14}C]5HT incorporation into the cells. As shown in Figure 4B, [^{14}C]5HT was dose dependent, with a V_{max} of 310 ± 36 pmol/30 min/mg proteins and a K_m of 5.9 ± 1.4 μM. [^{14}C]serotonin uptake was inhibited by the serotonin transport inhibitors clomipramine and fluoxetine with IC_{50} of 1 ± 0.4 nM and 10 ± 3.5 nM, respectively. In contrast, high concentrations of the nonselective monoamine transport inhibitors β estradiol (IC_{50} 3 ± 0.13 μM) and disprocynium 24 (IC_{50} 0.23 ± 0.1 μM) or of the biogenic amines norepinephrine (IC_{50} 1 μM) and dopamine (IC_{50} 1.5 mM) were required to block [^{14}C]5HT uptake (Fig. 5) [38]. This inhibition profile was similar to that previously reported in various tissues and species for the SERT.

Western blot analysis of the serotonin transporter expressed in mesangial cells

We definitively confirmed the expression of a SERT in MCs by Western blots analysis. Experiments were performed using a polyclonal antiserum directed against the SERT. Platelets that contained a SERT [28] were used as a positive control. As shown in Figure 6, in both platelets and MCs, the polyclonal antiserum revealed a peptide with an apparent molecular weight compatible with that reported for the SERT (approximately 70 kDa) [28]. Taken together, uptake experiments and Western blots show that MCs contain a SERT responsible for intracellular serotonin accumulation.

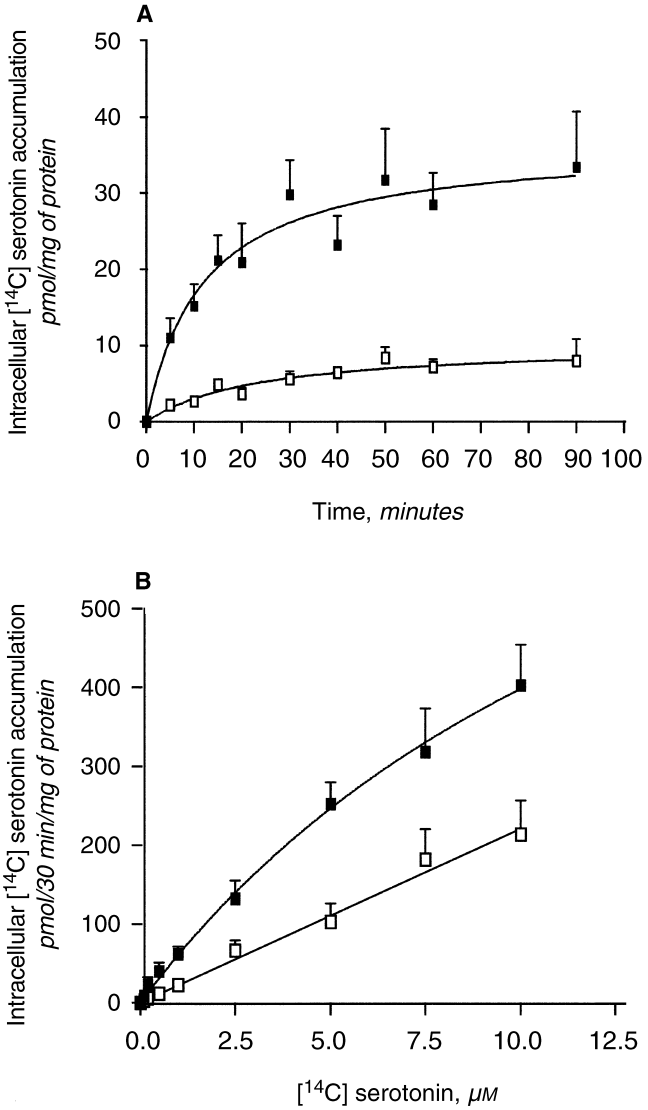

Fig. 4. [^{14}C]serotonin accumulation in rat mesangial cells. (*A*) Time course of [^{14}C]5HT accumulation. Total (■) and nonspecific (□) [^{14}C]5HT (200 nM) accumulation were measured as described in the **Methods** section. Nonspecific [^{14}C]5HT uptake was defined in presence of 10^{-5} M clomipramine. In order to inhibit MAOs, 10^{-5} M pargyline was added to the medium 20 minutes prior to the beginning of uptake experiments. All data represent the means ± SEM of four separate experiments. (*B*) Dose-dependent [^{14}C]5HT accumulation. Cells were incubated with various concentrations of [^{14}C]5HT at 37°C for 30 minutes in the absence (■) or in the presence of 10^{-5} M clomipramine (□). Monoamine oxidases were inhibited by 10^{-5} M of pargyline. All data represent the means ± SEM of seven separate experiments.

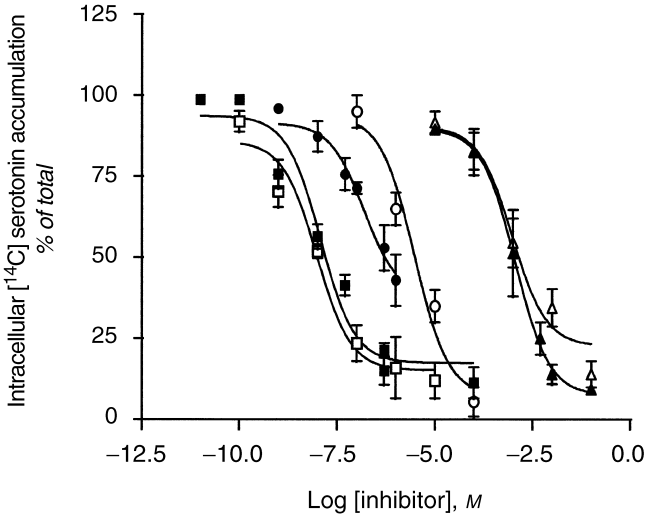

Fig. 5. Inhibition of [^{14}C]5HT uptake. Mesangial cells were incubated with [^{14}C]5HT (200 nM) in the absence or presence of the different inhibitors. The curves represent the means ± SEM of two to four separate experiments. Symbols are: (■) clomipramine; (□) fluoxetine; (●) disprocynium 24; (○) β-estradiol; (▲) norepinephrine; (△) dopamine.

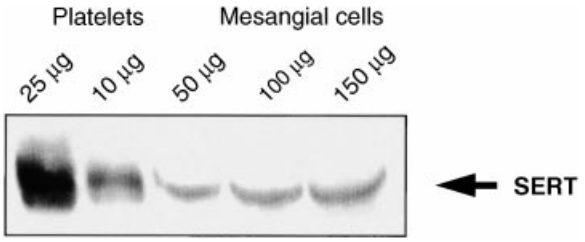

Fig. 6. Western blot of serotonin transporter (SERT). Crude extracts from rat mesangial cells and human platelets were electrophoresed on a 8% SDS-PAGE gel and transferred to polyvinylidene difluoride membranes. The blot was immunoblotted with SERT antibody (Santa Cruz Biotechnology). Immunoreactive proteins were identified as described in the **Methods** section. The arrow indicates immunoreactive protein with molecular mass of approximately 70 kDa. Data are representative of three independent experiments.

Intracellular metabolism of serotonin by rat mesangial cells

Next, we investigated whether serotonin, after the uptake into MCs, was metabolized by MAOs. With this attempt, we measured intracellular [^{14}C]5HT content and the release of serotonin metabolites in the extracellular medium with or without irreversible MAO inhibition. [^{14}C]Serotonin uptake experiments performed in the presence of the irreversible MAO inhibitor pargyline showed that [^{14}C]5HT accumulated into MCs in a time-dependent manner, and [^{14}C]5HT metabolites were slightly detectable in the extracellular medium (Fig. 7). In the absence of MAO inhibition, the amount of intracellular [^{14}C]5HT significantly decreased at the different time tested, and we observed a concomitant time-dependent release of [^{14}C]5HT metabolites in the extracellular medium (Fig. 7). These findings indicate that the decrease in intracellular radioactivity was related to the extracellular release of [^{14}C]5HT metabolites generated by MAO. These data show that after intracellular transport, serotonin is inactivated by MAO, and its metabolites are released in extracellular medium.

Effect of monoamine oxidase inhibition by pargyline on mesangial cell proliferation

As shown in Figure 8, serotonin induced a highly significant dose-dependent stimulation in BrdU incorporation, which reached the maximum (170 ± 11% increase) at 500 to 1000 nM. When experiments were performed in the presence of 10 µM pargyline, the serotonin-induced cell proliferation was enhanced significantly. A maximal 44 ± 8% increase was observed in the presence of 100 nM serotonin when compared with the effect induced by serotonin alone. No effect of pargyline alone on BrdU incorporation was observed.

DISCUSSION

Monoamine oxidase A and B are widely distributed in renal cortex and medulla. Most of the studies on renal MAOs concerned the proximal tubule. In this segment of the nephron, MAO-A is the predominant isoform, and it is involved in the regulation of the amount of dopamine and serotonin synthesized and released by the epithelial tubular cells [39, 40]. In the other renal cells, which are unable to synthesize dopamine and serotonin, intracellular transport systems are necessary to allow the substrate degradation by mitochondrial MAOs. Currently, very little is known concerning the expression and function of biogenic amines transport systems and MAO in the different portions of the nephron.

In this study, we show the expression of MAOs and a SERT in MCs. As shown by enzyme assays and Western blot analysis, MAO-A is the predominant isoform expressed in MCs and represents approximately 90% of the total MAO population. In contrast, the fact that MAO-B was significantly decreased in MCs as compared with whole renal cortex indicates that this enzyme isoform is mainly located in another cell type. MAO-A and MAO-B are generally considered specific for the degradation of serotonin and PEA, respectively. Surprisingly, we found that in MCs, MAO-A is able to metabolize PEA with an apparent affinity constant similar to that reported for PEA degradation by MAO-B [21]. In renal cortex, the ability of MAO-A to metabolize the MAO-B substrate seems to be specific of MCs. Indeed, enzyme assays performed in whole renal cortex and isolated proximal tubules showed that MAO-A and MAO-B display a classic substrate specificity (Copin et al, unpublished data) [21, 24]. It is noteworthy that, as

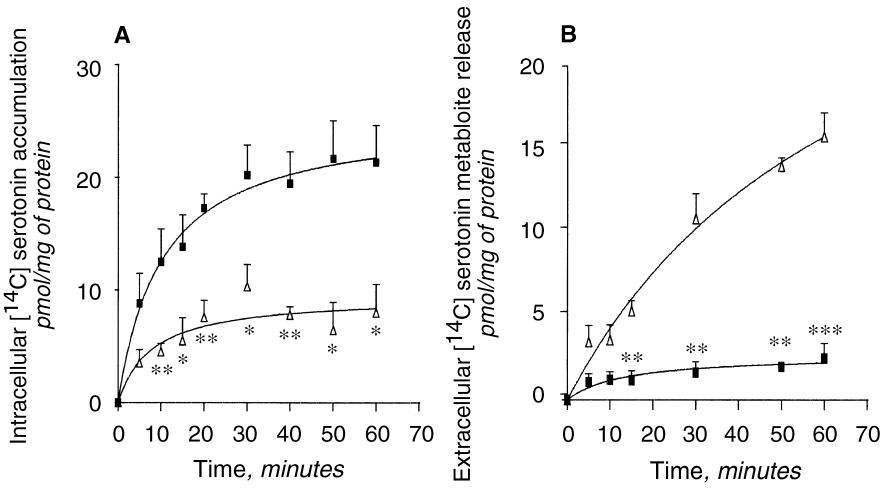

Fig. 7. Effect of monoamine oxidases on [^{14}C]5HT accumulation in rat mesangial cells. (*A*) Time course of intracellular [^{14}C]5HT accumulation. Specific accumulation of [^{14}C]-5HT (200 nM) was measured with (■) or without (△) the monoamine oxidase inhibitor pargyline (10^{-5} M). Specific transport was defined as the fraction of total uptake inhibited by 10^{-5} M of clomipramine. (*B*) Time course of extracellular [^{14}C]5HT metabolites accumulation. [^{14}C]Serotonin metabolites measured with (■) or without (△) the monoamine oxidase inhibitor pargyline (10^{-5} M). Experiments were performed as described in the **Methods** section. All data represent the means ± SEM of four separate experiments. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

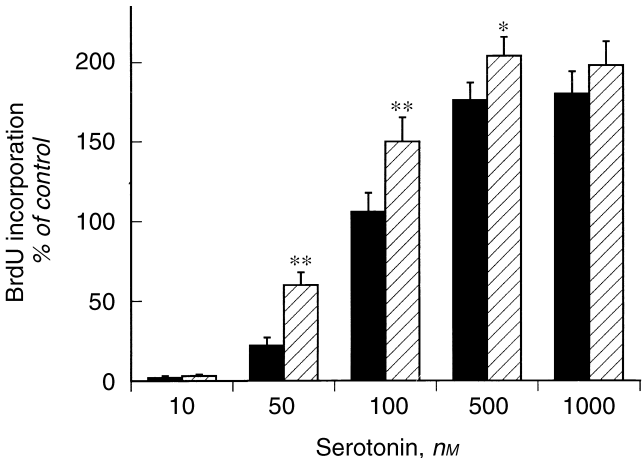

Fig. 8. Serotonin-dependent BrdU incorporation into mesangial cell (MC) entry into the S phase in the presence (▨) or absence (■) of the monoamine oxidase inhibitor pargyline. Quiescent MCs were stimulated with increasing doses of serotonin in the absence (■) and the presence of pargyline (▨). Results are means ± SEM of four distinct experiments. $*P < 0.05$; $**P < 0.01$.

observed in MCs, the rat heart MAO-A is also able to metabolize PEA (abstract; Guimaraes and Soares-Da-Silva, *Biochem Soc Trans* 25:S622, 1997). These data indicate that the classic substrate specificity of MAO-A and MAO-B is not absolute and may depend on cell-type–specific factors.

The demonstration of the expression of MAO-A in MCs suggested a potential role of this enzyme in the degradation of glomerular serotonin. This possibility has been further supported by our results showing the presence of a SERT in MCs. To date, the expression of a specific serotonin transport system in kidney was unknown. The studies of serotonin uptake and Western blot analysis showed that the SERT in MCs displays pharmacological and biochemical properties similar to those reported for SERTs identified in the central nervous system and in some peripheral cells or organs [25, 27, 28, 41]. This transporter displays a relative selectivity for serotonin as high concentrations of two other endogenous amines, norepinephrine and dopamine, were required to inhibit serotonin uptake. As shown by uptake experiments performed without MAO inhibition, MAO-A of MCs plays a major role in the degradation of extracellular serotonin. Indeed, in absence of the irreversible MAO inhibitor pargyline, we observed a concomitant decrease in intracellular serotonin accumulation and the release of serotonin metabolites in the extracellular medium. These results show that, as previously reported for presynaptic terminals and glial cells, SERT and MAO-A in MCs may play a critical role in removing extracellular serotonin.

The potential relevance of SERT and MAO-A in the regulation of MC functions is supported by our experiments on the effect of MAO inactivation on serotonin-mediated cell proliferation. Indeed, we showed that irreversible MAO inhibition significantly increased serotonin-mediated BrdU incorporation into MCs. It is noteworthy that the effect of MAO inactivation was maximal at 50 and 100 nM serotonin, concentrations close to those found in plasma of diabetic patients [42, 43]. Serotonin is one of the factors involved in the development and progression of glomerulonephritis at least through two distinct mechanisms: the proliferation of MCs and the production of type IV collagen [44]. It is generally acknowledged that the serotonin concentration in glomeruli depends mainly on platelet release. In contrast, our results show that serotonin concentration and function in glomeruli may be also regulated by its uptake and degradation by MCs. Therefore, SERT and MAO-A may play a protective role against the deleterious effects of serotonin on glomeruli, and it is conceivable that their

impairment may be involved in abnormalities of MC function under pathological situations.

In conclusion, the demonstration of the coexpression of a SERT and MAO-A in MCs opens new perspectives for the comprehension of the mechanisms involved in the development of serotonin-mediated glomerular diseases and the basis for potential novel therapeutical approaches of these pathologies.

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APPENDIX

Abbreviations used in this article are: α-SMA, α smooth muscle actin; FCS, fetal calf serum; 5HT, 5 hydroxytryptamine (serotonin); MAO, monoamine oxidase; MC, mesangial cell; PEA, β-phenylethylamine; SDS, sodium dodecyl sulfate, and SERT, serotonin transporter.

REFERENCES

1. Schlondorff D: The glomerular mesangial cell: An expanding role for a specialized pericyte. *FASEB J* 1:272–281, 1987
2. Floege J, Johnson RJ, Gordon K, Lida H, Pritzl P, Yoshimura A, Campbell C, Alpers CE, Couser WG: Increased synthesis of extracellular matrix in mesangial proliferative nephritis. *Kidney Int* 40:477–488, 1991
3. Sterzel RB, Schulze-Lohoff E, Marx M: Cytokines and mesangial cells. *Kidney Int* 41(Suppl 39):S26–S31, 1993
4. Barnett R, Singhal PC, Scharschmidt LA, Schlondorff D: Dopamine attenuates the contractile response to angiotensin II in isolated rat glomeruli and cultured mesangial cells. *Circ Res* 59:529–533, 1986
5. Garcia-Escribano C, Diez-Marques ML, Gonzalez-Rubio M, Rodriguez-Puyol M: Somatostatin antagonizes angiotensin II effects on mesangial cell contraction and glomerular filtration. *Kidney Int* 43:324–333, 1993
6. Bascands JL, Pecher C, Bompart G, Rakotoaryvony J, Leung-Tack J, Girolami JP: Bradykinin-induced *in vitro* contraction of rat mesangial cells *via* a B2 receptor type. *Am J Physiol* 267:F871–F878, 1994
7. Montero A, Rodriguez-Lopez A, Reverte M, Lopez-Novoa JM: The effect of LU-49938 (nexopamil) on the activation by serotonin of mesangial cells. *Fund Clin Pharmacol* 9:557–566, 1995
8. Kanai H, Hiromura K, Kuroiwa T, Maezawa A, Yano S, Naruse T: Role of serotonin in nephrotoxic serum nephritis in WKY rats. *J Lab Clin Med* 129:557–566, 1997
9. Radeke HH, Resch K: The inflammatory function of renal glomerular mesangial cells and their interaction with the cellular immune system. *Clin Invest* 70:825–842, 1992
10. Fanburg BL, Lee SL: A new role for an old molecule: Serotonin as a mitogen. *Am J Physiol* 272:L795–L806, 1997
11. Takuwa N, Ganz M, Takuwa Y, Sterzel RB, Rasmussen H: Studies of the mitogenic effect of serotonin in rat renal mesangial cells. *Am J Physiol* 257:F431–F439, 1989
12. Kurscheid-Reich D, Throckmorton DC, Rasmussen H: Serotonin activates phospholipase D in rat mesangial cells. *Am J Physiol* 268:F997–F1003, 1995
13. Lee SL, Wang WW, Fanburg BL: Superoxide as an intermediate signal for serotonin-induced mitogenesis. *Free Radic Biol Med* 24:855–858, 1998
14. Lee SH, Wang WW, Fanburg BL: Association of Tyr phosphorylation of GTPase-activating protein with mitogenic action of serotonin. *Am J Physiol* 272:C223–C230, 1997
15. Soares-Da-Silva P, Pinto-do-O PC, Bertorello AM: Antagonistic actions of renal dopamine and 5-hydroxytryptamine: Increase in Na^+,K^+-ATPase activity in renal proximal tubules via activation of $5\text{-}HT_{1A}$ receptors. *Br J Pharmacol* 117:1199–1203, 1996
16. Tuomainen P, Törnwall M, Männistö PT: Minor effect of tolcapone, a catechol-O-methyltransferase inhibitor, on extracellular dopamine levels modified by amphetamine or pargyline: A microdialysis study in anaesthetized rats. *Pharmacol Toxicol* 78:392–396, 1996
17. Pastorova B, Varady J: Catecholamine levels and activity of monoamine oxidase in some hypothalamic structures and in the pineal gland of sheep after administration of FSH. *Physiol Res* 45:131–136, 1996
18. Grimsby J, Chen K, Wang L, Lan N, Shih J: Human monoamine oxidase A and B genes exhibit identical exon-intron organization. *Proc Natl Acad Sci USA* 88:3637–3641, 1991
19. Weyler W, Hsu Y, Breakefield X: Biochemistry and genetics of monoamine oxidase. *Pharmacol Ther* 47:391–417, 1990
20. Youdim MBH, Finberg JPM: New directions in monoamine oxidases A and B selective inhibitors and substrates. *Biochem Pharmacol* 41:155–162, 1991
21. Fernandes MH, Soares-Da-Silva P: Type A and B monoamine oxidase activities in the human and rat kidney. *Acta Physiol Scand* 145:363–367, 1992
22. Saura J, Kettler R, Da Prada M, Richards JG: Quantitative enzyme radioautography with ^{3}H-Ro 41-1049 and ^{3}H-Ro 19-6327 *in vitro*: Localization and abundance of MAO-A and MAO-B in rat CNS, peripheral organs, and human brain. *J Neurosci* 12:1977–1999, 1992
23. Gargalidis-Moudanos C, Remaury A, Pizzinat N, Parini A: Predominant expression of monoamine oxidase B isoform in rabbit renal proximal tubule: Regulation by I_2 imidazoline ligands in intact cells. *Mol Pharmacol* 51:637–643, 1997
24. Guimaraes JT, Soares-Da-Silva P: The activity of MAO-A and B in rat renal cells and tubules. *Life Sci* 62:727–737, 1998
25. Blakely RD, De Felice LJ, Hartzell HC: Molecular physiology of norepinephrine and SERTs. *J Exp Biol* 196:263–281, 1994
26. Bel N, Figueras G, Vilaro MT, Sunol C, Artigas F: Antidepressant drugs inhibit a glial 5-hydroxytryptamine transporter in rat brain. *Eur J Neurosci* 9:1728–1738, 1997
27. Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V, Blakely RD: Antidepressant- and cocaine-sensitive human SERT: Molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci USA* 90:2542–2546, 1993
28. Worrall M, Williams C: Sodium ion-dependent transporters for neurotransmitters: A review of recent developments. *Biochem J* 297:425–436, 1994
29. Balkovezt DF, Tiruppathi C, Leibach FH, Mahesh VB, Ganapathy V: Evidence for an imipramine-sensitive SERT in human placental brush-border membranes. *J Biol Chem* 28:2195–2198, 1989
30. Blakely RD, Berson HE, Fremeau RT Jr, Caron MG, Peek MM, Prince HK, Bradley CC: Cloning and expression of a functional serotonin transporter from rat brain. *Nature* 354:66–70, 1991
31. Edmond C, Bascands JL, Pecher C, Cabos-Boutot G, Pradelles P, Regoli D, Girolami JP: Characterization of B2 bradykinin receptor in rat mesangial cells. *Eur J Pharmacol* 190:381–392, 1990
32. Yu PH: Monoamine oxidase, in *Neuromethods, Neurotransmitter Enzymes*, edited by Boulton AA, Baker GB, Yu PH, Clifton, Human Press, 1986, pp 235–272
33. Wurtman RJ, Axelrod J: A sensitive and specific assay for estimation of monoamine oxidase. *Biochem Pharmacol* 12:1437–1454, 1963
34. Singer TP, Ramsay RR: New aspects of the substrate specificities, kinetic mechanisms and inhibition of monoamine oxidases, in *Monoamine Oxidases: Basic and Clinical Aspects*, edited by Yasuhara H, Parvez SH, Oguchi K, Sandler M, Nagatsu T, Utrecht, VSP, 1993, pp 23–48
35. Lanier B, Raddatz R, Bakthavachalam V, Coupry I, Neumeyer JL, Lanier SM: Structural and ligand recognition properties of

imidazoline binding proteins in tissues of rat and rabbit. *Mol Pharmacol* 48:703–710, 1995
36. Raddatz R, Parini A, Lanier SM: Localization of the imidazoline binding domain on monoamine oxidase B. *Mol Pharmacol* 52:549–553, 1997
37. Pizzinat N, Copin N, Vindis C, Parini A, Cambon C: Reactive oxygen species production by monoamine oxidases in intact cells. *Naunyn Schmiedbergs Arch Pharmacol* 359:428–431, 1999
38. Russ H, Sonna J, Keppler K, Baunach S, Schömig E: Cyanine-related compounds: A novel class of potent inhibitors of extraneuronal noradrenaline transport. *Naunyn Schmiedebergs Arch Pharmacol* 348:458–465, 1993
39. Fernandes MH, Soares-Da-Silva P: Role of monoamine oxidase and cathecol-O-methyltransferase in the metabolism of renal dopamine. *J Neural Transm* 41(Suppl):101–105, 1994
40. Soares-Da-Silva P, Vieira-Coelho MA, Pestena M: Antagonistic actions of renal dopamine and 5-hydroxytryptamine: Endogenous 5-hydroxyprytptamine, 5-HT_{1A} receptors and antinatriuresis during high sodium intake. *Br J Pharmacol* 117:1193–1198, 1996
41. Wolfel R, Graefe K-H: Evidence for various tryptamines and related compounds acting as substrates of the platelet 5-hydroxytryptamine transporter. *Naunyn Schmiedebergs Arch Pharmacol* 345:129–136, 1992
42. Barradas MA, Gill DS, Fonseca VA, Mikhailidis DP, Dandona P: Intraplatelet serotonin in patients with diabetes mellitus and peripheral vascular disease. *Eur J Clin Invest* 18:399–404, 1988
43. Malysko J, Urano T, Knofler R, Taminato A, Takada Y, Takada A: Daily variations of platelet aggregation in relation to blood and plasma serotonin in diabetes. *Thromb Res* 75:569–576, 1994
44. Kasho M, Sakai M, Sasahara T, Anami Y, Matsumura T, Takemura T, Matsuda H, Kobori S, Shichiri M: Serotonin enhances the production of type IV collagen by human mesangial cells. *Kidney Int* 54:1083–1092, 1998